

Proerectile Pharmacological Effects of *Tribulus terrestris* Extract on the Rabbit Corpus Cavernosum

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Abstract

Introduction: The objective of the present study was to investigate the effect of oral treatment of *Tribulus terrestris* (TT) extract on the isolated corpus cavernosal tissue of New Zealand white (NZW) rabbits and to determine the mechanism by which protodioscin (PTN), a constituent of the TT, exerts its pharmacological effects. **Materials and Methods:** Twenty-four NZW rabbits were randomly assigned to 4 experimental groups of 6 each. Group I served as control. Groups II to IV were treated with the extract at different dose levels, i.e. 2.5 mg/kg, 5 mg/kg and 10 mg/kg body weight, respectively. The TT extract was administered orally, once daily, for a period of 8 weeks. The rabbits were then sacrificed and their penile tissue isolated to evaluate the responses to both contracting and relaxing pharmacological agents and electrical field stimulation (EFS). **Results:** PTN on its own had no effect on the isolated corpus cavernosal strips. The relaxant responses to EFS, acetylcholine and nitroglycerin in noradrenaline precontracted tissues from treated group showed an increase in relaxation of a concentration dependent nature compared to that of the tissues from control group. However, the contractile, anti-erectile response of corpus cavernosal tissue to noradrenaline and histamine showed no significant change between the treatment and the control groups. **Conclusions:** The relaxant responses to acetylcholine, nitroglycerin and EFS by more than 10%, 24% and 10% respectively compared to their control values and the lack of such effect on the contractile response to noradrenaline and histamine indicate that PTN has a proerectile activity. The enhanced relaxant effect observed is probably due to increase in the release of nitric oxide from the endothelium and nitrergic nerve endings, which may account for its claims as an aphrodisiac. However, further study is needed to clarify the precise mechanism of its action.

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Key words: Electrical field stimulation, Nitric oxide, Protodioscin, Rabbit corpus cavernosum, *Tribulus terrestris*

Introduction

Erectile dysfunction (ED) is the most common sexual problem in men after premature ejaculation. ED, which usually has psychogenic and organic causes, is defined as the inability to achieve or maintain an erection for satisfactory sexual performance.¹ Penile erection is a complex physiologic event requiring the interaction of central and peripheral nervous system and end organ neurotransmitters. Penile tumescence and erection result from integration and co-ordination of three processes namely arteriolar dilatation, relaxation of the smooth muscle within the corpora cavernosa and corporeal veno-occlusion.² Although great progress has been made in the diagnosis and treatment of erectile failure, an effective oral medication, without any systemic adverse effect, is still awaited.

Tribulus terrestris (TT) Linn (Zygophyllaceae), popularly known as puncture vine, is a perennial creeping herb, with a worldwide distribution. Since ancient times, it is regarded as an aphrodisiac in addition to its other beneficial effects on various ailments such as urinary infections, inflammations, leucorrhoea, oedema and ascites.^{3,4} The extract obtained from the air-dried aerial parts of the plant contains steroidal glycosides (saponins) of furastanol type, isolated by preparative column chromatography on silica gel and elution with CHCl₃-CH₃OH-H₂O, the predominant active component being protodioscin (PTN), which is as much as 45% of the extract.^{5,6} A report from Bulgaria indicates that the administration of this extract to humans and animals improves libido and spermatogenesis.⁶ It is claimed to dilate coronary arteries and improve coronary

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perfusion with no adverse reaction on long-term use.⁷ Testosterone and leutinising hormones are also said to be enhanced following treatment for erectile dysfunction with PTN for a period of 30 to 90 days.⁸ The administration of PTN is found to be safe both in animals and humans following both acute and chronic toxicity studies. Furthermore, PTN treatment has been shown to improve dehydroepiandrosterone (DHEA) level in man.⁹ While the plant or its products are being consumed by people in different parts of the world essentially for its claims as an aphrodisiac, there is no systematic scientific study to validate its effect on the mechanisms of erection.

In this preliminary report of our series of studies on TT extract as an aphrodisiac, we validate its *in vitro* pharmacological effects on the corpus cavernosal (CC) tissue of rabbit after oral treatment for 8 weeks.

Materials and Methods

Adult male New Zealand white (NZW) rabbits (2.5 to 3 kg body weight) were chosen as the animal model because close similarities exist in the reactivity of human and rabbit corpus cavernosum.¹⁰ Twenty-four NZW rabbits were randomly divided into 4 experimental groups of 6 animals each. Group I was treated with the vehicle (distilled water) and served as the control while Group II, Group III and Group IV were treated with the aqueous extract of TT orally, at 2.5 mg/kg, 5 mg/kg and 10 mg/kg body weight, respectively, in a once daily regimen for a period of 8 weeks. The rabbits had ready access to rabbit pellets and water *ad libitum*. The pellets were made up of mainly barley, oats, lupins, millmix, soya meal, lucerene, limestone, methionine, salt and added vitamins and mineral premix. The rabbits were then sacrificed with an inhalational overdose of carbon dioxide and underwent surgical excision of the penis. The corpora cavernosa were carefully separated out from the tunica albuginea, and sectioned transversely producing two strips (0.2 x 0.2 x 1.0 cm) from each corpus.

In vitro Experiments

The strips were mounted in 25 mL organ bath chambers containing freshly prepared Tyrode solution, aerated with 95% oxygen and 5% carbon dioxide. The pH was maintained at 7.4 and temperature at 37°C. The smooth muscle strips were allowed to equilibrate for 60 minutes at the organ bath conditions with an initial tension of 1.5 g before the addition of test compounds.

Concentration-response curves to contractions of noradrenaline and histamine were obtained by cumulative additions of the respective agents. Relaxant responses to acetylcholine and nitroglycerin of the corpus cavernosum were assessed using precontracted strips with EC₅₀ of noradrenaline.

Electrical Field Stimulation (EFS)

In organ bath chambers, the CC strips were mounted between the two parallel platinum wire electrodes (25 mm long and 8 mm apart). The strips were stimulated for 10 seconds at sequential frequencies of 2, 5, 10, 20 and 40 Hz as square wave pulses of 90 v, 1 ms duration using Grass stimulator (S88).¹¹ Before EFS, the tissue was treated with adrenergic neurone blocker, guanethidine (5.04 µM) and a muscarinic receptor blocker, atropine (3.45 µM) for 30 minutes to eliminate the adrenergic and cholinergic responses and to study exclusively the relaxation response induced by stimulation of the nonadrenergic noncholinergic nerves (NANC).

Drugs

The following drugs were used: noradrenaline, histamine, acetylcholine, N-nitro-L-arginine (Sigma), nitroglycerin (Schwarz Pharma), atropine (Astra), guanethidine (Ciba-Geigy) and TT extract (Sopharma, Bulgaria & Teguhindo, Indonesia).

Stock solution of the drugs were stored at -20°C and diluted to the required concentration with physiological saline (0.9% w/v) just prior to use, on the day of experimentation. The drug concentration was expressed as the final level of the agent in organ bath fluid.

Analysis of Data

Statistical analysis of the data was performed and the results expressed as mean ± standard error of the mean (SEM) with “n” representing the number of strips. Multi-variate analysis of variance and Student’s *t*-tests for independent samples were used to determine the significant differences between the treated and control groups. Statistical significance was considered when *P* < 0.05.

Results

The cumulative addition of noradrenaline and histamine produced concentration-dependent contractions of the CC strips. The noradrenaline concentration response curve showed slight inhibition of contraction in the treated groups (Groups II, III and IV) (Fig. 1). However, there was no statistically significant inhibition of noradrenaline contraction to increasing doses of the test drug when compared to its control responses. Histamine also showed inhibition of contraction in the treated group although not significant statistically (Fig. 2).

In the presence of active muscle tone induced by noradrenaline (9.46M), the CC strips of the control group (Group I) relaxed immediately upon addition of acetylcholine (ACh) and nitroglycerin in a concentration dependent manner and the results are expressed as percentage of noradrenaline-induced tone. The ACh response showed statistically significant enhancement of

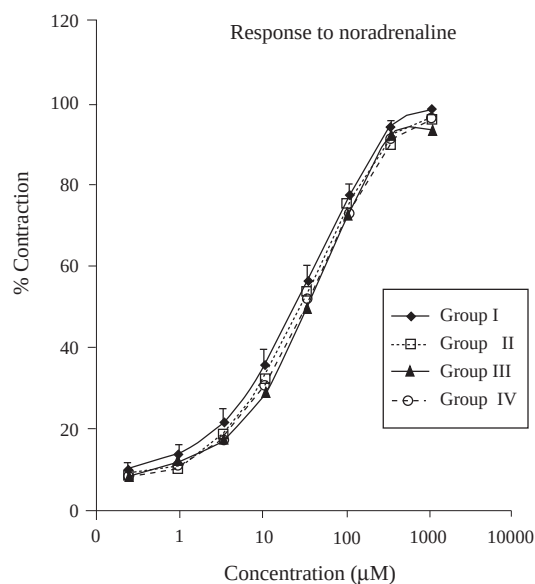


Fig. 1. Contractile responses of rabbit corpus cavernosum strips to noradrenaline in control (Group I) and *Tribulus terrestris* (TT)-treated groups (Group II–TT 2.5 mg/kg, Group III–TT 5 mg/kg and Group IV–TT 10 mg/kg body weight, respectively). Contractile responses are expressed as percentage of maximum contraction and compared among the control and treatment groups. Values are expressed as mean $\pm$ SEM for 20 to 25 strips from 6 rabbits.

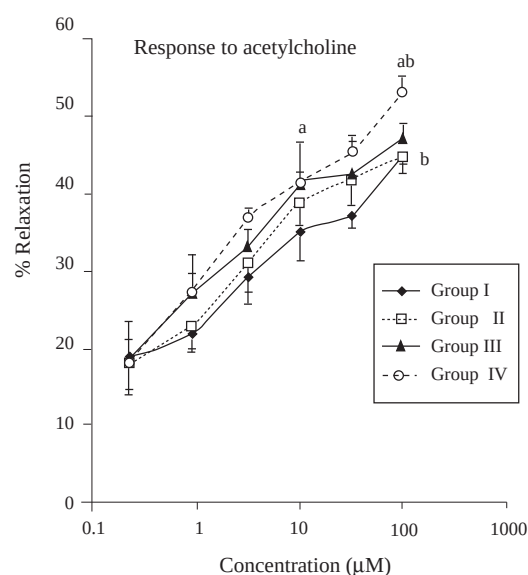


Fig. 3. Relaxant responses to acetylcholine on noradrenaline precontracted rabbit corpus cavernosum smooth muscle in control rabbits (Group I) and *Tribulus terrestris* (TT)-treated groups (Group II–TT 2.5 mg/kg, Group III–TT 5 mg/kg and Group IV–TT 10 mg/kg body weight, respectively). The relaxant responses are expressed as percentage of noradrenaline-induced tone and compared among the control and treatment groups. Values are expressed as mean $\pm$ SEM for 10 to 14 strips from 6 rabbits. a and b indicate significant differences ($P < 0.05$) from Groups I and II, respectively.

relaxation in Group IV, reaching a maximum of 11% and 8% at lower concentrations of ACh compared to Groups I and II. Group III also showed enhancement of relaxation but this was not statistically significant compared to the control group (Fig. 3). The response to nitroglycerin showed an enhancement of relaxation in Group IV, which was

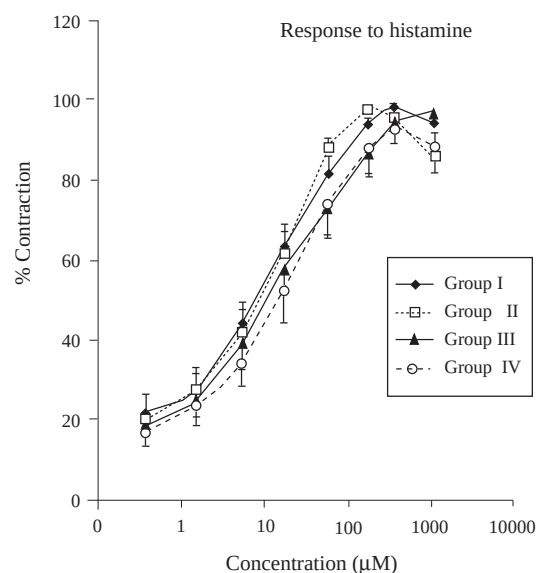


Fig. 2. Contractile responses of rabbit corpus cavernosum strips to histamine in control (Group I) and *Tribulus terrestris* (TT)-treated groups (Group II–TT 2.5 mg/kg, Group III–TT 5 mg/kg and Group IV–TT 10 mg/kg body weight, respectively). Contractile responses are expressed as percentage of maximum contraction and compared among the control and treatment groups. Values are expressed as mean $\pm$ SEM for 6 to 9 strips from 6 rabbits.

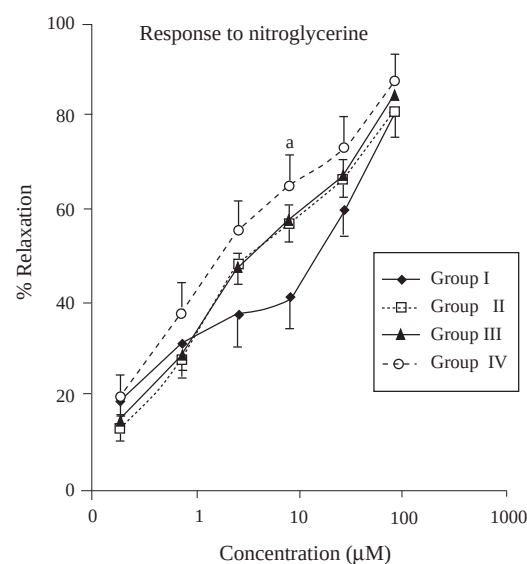


Fig. 4. Relaxant responses to nitroglycerin on noradrenaline precontracted rabbit corpus cavernosum smooth muscle in control rabbits (Group I) and *Tribulus terrestris* (TT)-treated groups (Group II–TT 2.5 mg/kg, Group III–TT 5 mg/kg and Group IV–TT 10 mg/kg body weight, respectively). The relaxant responses are expressed as percentage of noradrenaline-induced tone and compared among the control and treatment groups. Values are expressed as mean $\pm$ SEM for 10 to 14 strips from 6 rabbits. a indicates significant differences ($P < 0.05$) from control.

significant (24% increase compared to the control group) at concentrations ranging from 2.47 μ M to 78.21 μ M (Fig. 4). Groups II and III also showed enhancement in relaxation but this was not significant compared to the control group.

EFS elicited relaxation of the CC strips in a frequency dependent manner and the results are expressed as

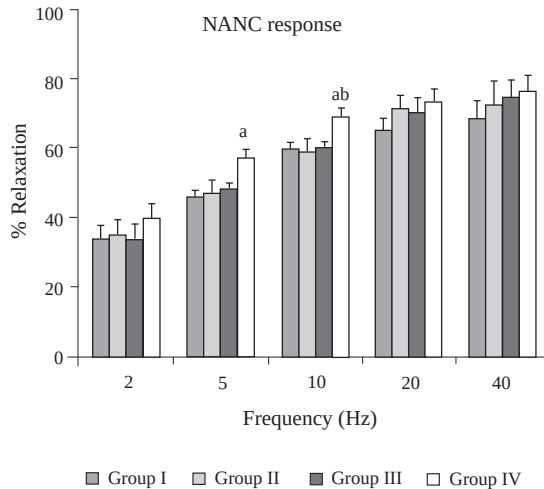


Fig. 5. Relaxation responses to EFS at 90 v, 1 ms and 10 s duration at different frequencies on rabbit corpus cavernosum strips preincubated for 30 minutes with atropine ($3.45\mu\text{M}$) and guanethidine ($5.04\mu\text{M}$) precontracted with noradrenaline in control rabbits (Group I) and *Tribulus terrestris* (TT)-treated groups (Group II–2.5 mg/kg, Group III–5 mg/kg and Group IV–10 mg/kg body weight, respectively). The relaxations expressed as percentage of noradrenaline-induced tone and compared among the control and treatment groups. Values are expressed as mean $\pm$ SEM for 10 to 14 strips from 6 rabbits. a and b indicate significant differences ($P < 0.05$) from the respective control.

percentage of noradrenaline-induced tone (Fig. 5). The relaxation response in Group IV showed significant increase of about 10% compared to Groups I and II. Group III also showed an enhancement in relaxation but this was not statistically significant. The relaxations induced by EFS at various frequencies were abolished by N-nitro-L-arginine ($50.2\mu\text{M}$, data not shown).

Discussion

There are at least three neuroeffector systems that control trabecular corpus cavernosal smooth muscle tone, namely, adrenergic (antiectile), cholinergic and nonadrenergic noncholinergic-NANC neurotransmissions (nitrgergic). The state of detumescence is maintained by the sympathetic nervous system stimulating post-junctional α -1 adrenoceptors through continuous release of noradrenaline into the synaptic cleft.¹² In the present study, it is observed that there is a minimal inhibitory effect on the contractile response to noradrenaline, which was not statistically significant. Histamine produces contraction of rabbit CC muscle *in vitro* and the H_1 receptor is responsible for the contractile effect which is antagonised by the selective H_1 receptor antagonist, mepyramine.¹³ In this present study, histamine produced a minimal inhibitory effect on the contractile response although not significant statistically. Thus, by not promoting, but mildly inhibiting the antiectile responses of α -1 adrenoceptor and histamine H_1 receptors of the cavernosum, PTN could positively contribute to the proerectile physiology of the cavernosum (see below).

Corporal smooth muscle relaxation and an increase in corporeal blood flow are necessary for penile tumescence and rigidity.¹⁴ Both the muscarinic receptor stimulation and activation of the nitrgergic neurotransmission are involved in the relaxation mechanism. Nitric oxide (NO) released from the endothelium and the cavernous nerves in the penis causes erectile smooth muscle relaxation through the release of cGMP. *In vitro* studies using EFS on rabbit corpus cavernosal tissue during muscarinic and adrenergic blockade have elicited NANC neurogenic relaxation. This effect was blocked by NO synthase inhibitors indicating that NO is required for the response and it is the NANC neurotransmitter.¹² It is also known that the cholinergic stimulation of the muscarinic receptors on the endothelial cells release NO, which aids in cavernosal smooth muscle relaxation.¹⁵ In the present study, it is demonstrated that long-term treatment of TT extract enhanced acetylcholine, nitroglycerine and EFS elicited relaxations in the corpus cavernosum of the rabbits. The effects of TT extract probably appear to be mediated by release and/or the modification of release of NO from endothelial cells and nitrgergic nerve endings.

Cavernosal response to ACh significantly increased following testosterone replacement in castrated animals.¹⁶ Nitric oxide synthesis in penis seems to depend on testosterone levels in rats. Castration produced 45% decrease in nitric oxide synthesis in the rat penis and this was prevented by testosterone replacement at the time of surgery.¹⁷ Also the lower levels of testosterone and nitric oxide in the penile venous blood compared to brachial venous blood in patients with psychogenic impotence support the testosterone dependence of penile nitric oxide synthesis.¹⁸ Thus, the influence of androgens on nitric oxide synthesis within the male reproductive tract may play a contributory role in the erectile physiology. Protodioscin is also known to increase the level of testosterone⁸ and DHEA.⁹ It is therefore pertinent to consider the possibility of an additional pathway whereby PTN could increase the intracellular NO through increasing testosterone levels secondary to PTN intake.

In conclusion, the results of the present study indicate that long-term treatment of PTN in rabbit increases the sensitivity of the CC to NO releasing agents and nitrgergic neurotransmission. In addition, PTN may also exert its effects on NO release through the steroidal pathway by increasing the level of testosterone/DHEA. Further studies are being conducted to validate this hypothesis.

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